Magna Scientia Advanced Biology and Pharmacy

eISSN: 2582-8363

Cross Ref DOI: 10.30574/msabp

Journal homepage: https://magnascientiapub.com/journals/msabp/

(RESEARCH ARTICLE)

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Toxicity of cocoa bean shell extract (*Theobroma cacao* L.) on the mortality of A*edes aegypti* L. Mosquito Larvae

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Magna Scientia Advanced Biology and Pharmacy, 2025, 15(01), 016-020

Publication history: Received on 26 March 2025; revised on 03 May 2025; accepted on 06 May 2025

Article DOI: https://doi.org/10.30574/msabp.2025.15.1.0037

Abstract

The A*edes aegypti* L. mosquito is one of the vectors of Dengue Hemorrhagic Fever. Control of mosquito vectors is expected to have an impact on reducing the population of the mosquito vector A*edes aegypti* L. so that it is no longer significant as a disease transmitter. One way to control this mosquito vector is by using biolarvicide which comes from the cocoa bean shell. The aim of this research was to determine the toxicity (LC50) of cocoa bean shell extract on the mortality of A*edes aegypti* L. mosquito larvae. The research subjects were 640 A*edes aegypti* L. larvae divided into eight treatment groups. The concentrations used in this research were 100 ppm, 400 ppm, 700 ppm, 1000 ppm, 1300 ppm, 1600 ppm, positive control used abate and negative control used distilled water. Observations were carried out within 24 hours. Calculation of the Lethal Concentration 50 (LC50) value used probit analysis. The results of the probit analysis showed that the LC50 value was at a concentration of 787.14 ppm. Cocoa bean shell extract (Theobroma cacao L.) is toxic to A*edes aegypti* L. larvae at a concentration of 787.14 ppm. Conclusion: Cocoa bean shell extract (Theobroma cacao L.) at a concentration of 787.14 ppm was able to kill 50% of A*edes aegypti* L. larvae within 24 hours

Keywords: *Aedes aegypti* L; Cocoa Bean; Shell Extract; Mosquito Larvae

1. Introduction

Mosquitoes are one of the vectors that transmit diseases, such as Dengue Hemorrhagic Fever (DHF), which is caused by the A*edes aegypti* L. mosquito. Currently, the control of A*edes aegypti* L. largely depends on the use of larvicides such as abate (temephos), a synthetic organic chemical that can kill mosquito larvae quickly and cover a wide area. However, the use of abate (temephos) has several drawbacks. These include the potential for insect resistance, the poisoning of some birds, bees, and aquatic animals, as well as environmental pollution and insecticide residues. To overcome the problem of excessive use of synthetic insecticides, natural alternatives can be employed by utilizing plants or botanical insecticides—commonly known as biolarvicides—which are safer and more selective (Ekawati *et al*., 2017).

Natural insecticides derived from plants have the potential to control vectors, whether in eradicating larvae or adult mosquitoes. These natural insecticides are biodegradable and do not cause long-term environmental pollution; they are relatively safe for humans, the ecosystem, and animals because their residues disappear quickly. Their lethal effect is due to toxic compounds inherent in the substance, acting as respiratory toxins, contact toxins, or stomach toxins on soft-bodied organisms. Hence, an alternative in the form of natural or botanical larvicides made from plant materials is needed for effective control (Saleh *et al*., 2017).

Cocoa bean shells (Theobroma cacao L.) are the thin, soft, and slightly mucilaginous covering of the cocoa nib. They account for about 10–16% of the total dry weight of the cocoa bean. Up to now, cocoa bean shells have not been optimally utilized and are considered to have low economic value. They are typically used for animal feed or compost.

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However, cocoa bean shells have the potential to serve as botanical larvicides because they are known to contain secondary metabolites such as flavonoids, alkaloids, steroids, saponins, and tannins (Dewi et al., 2021).

2. Methodology

The study on the toxicity of cocoa bean shell extract (Theobroma cacao L.) against the mortality of A*edes aegypti* L. mosquito larvae was an experimental laboratory study using a Completely Randomized Design (CRD), conducted at the Toxicology Laboratory of the Biology Education Program, Faculty of Teacher Training and Education, University of Jember. The research was carried out from January to June 2023.

2.1. Apparatus and Materials

The apparatus used in this research included: plastic trays for rearing larvae analytical balance, blender, plastic jars, stirring spoons, sieve and filter paper, funnel, graduated cylinder, rotary evaporator, spatula, jam jars, glass plates, oven, refrigerator, tissue paper, camera, label paper, pipettes, aluminum foil, plastic glasses. The materials included: cocoa bean shell extract (Theobroma cacao L.), 97% Ethanol, A*edes aegypti* L. larvae (late instar III to early instar IV), tween 20, abate (positive control), distilled water (negative control).

2.2. Larvae Identification

Larvae were identified both microscopically and macroscopically. Microscopically, identification was done by observing larval morphology such as size, color, anal side teeth bristles, and the respiratory siphon. Macroscopically, identification was conducted by observing the larval resting phase.

2.3. Ekstract Preparation

After larvae identification, the next step was extract preparation. The cocoa bean shells, which had been separated from the cocoa nibs, were air-dried. Once dried, the shells were ground using a blender until they became a fine powder. The powder was weighed and transferred to a jar, and 97% ethanol was added at a ratio of 1:3. The mixture was stirred with a spatula or spoon until homogeneous and then tightly sealed. The extraction was performed by maceration for 3 days with manual stirring every 6 hours. The mixture was then filtered using filter paper and a funnel. The filtrate was evaporated using a rotary evaporator at 50°C for approximately 3 hours at 50 rpm. Finally, the concentrated residue was placed in an oven at 40°C for about 24 hours until a thick, concentrated extract was obtained. The extract was then stored in a refrigerator until it was applied in the test.

2.4. Toxicity Test of Cocoa Bean Shell (*Theobroma cacao* L.)

The toxicity test was conducted using several serial concentrations of the extract: 100 ppm, 400 ppm, 700 ppm, 1000 ppm, 1300 ppm, and 1600 ppm. In addition to these extract concentrations, positive control (abate) and negative control (distilled water) were used. Each treatment was repeated four times with 20 larvae per treatment.

The test began by introducing 20 larvae (using a dropper) into a plastic cup containing 100 mL of water mixed with the extract at the predetermined concentration. After 24 hours, the movement and mortality of the larvae were observed by gently touching the larvae with a pipette. The number of dead larvae was then recorded for each treatment, and the LC_{50} value was determined using probit analysis with SPSS software.

3. Result

Based on the research results, the average mortality rate of A*edes aegypti* L. mosquito larvae treated with cocoa bean shell extract (Theobroma cacao L.) is shown in Table 1.

Table 1 Final test results of cocoa bean shell extract (Theobroma cacao L.) on the mortality of A*edes aegypti* L. mosquito larvae after 24 hours of exposure

Treatment/Control	**Mosquito larval mortality *Aedes aegypti* L. (%)**				**Average (%)**
	Replication				
	1	2	3	4	
C-	0	0	0	0	0
C+	100	100	100	100	100
100 ppm	5	0	5	5	3,75
400 ppm	15	15	15	20	16,25
700 ppm	45	40	40	45	42,5
1000 ppm	65	70	65	65	66,25
1300 ppm	80	80	80	85	81,25
1600 ppm	95	95	90	95	93,75

Table 1 shows that as the concentration of cocoa bean shell extract increases, the percentage of larval mortality also increases. The negative control (distilled water) did not cause any larval death after 24 hours, whereas the positive control (abate at 100 ppm) caused 100% mortality in 24 hours.

Tabel 1 Probit analysis of LC_{50} for cocoa bean shell extract (Theobroma cacao L.) on A*edes aegypti* L. mosquito larvae after 24 hours of exposure

Lethal Concentration 50% (LC_{50})	**Concentration (ppm)**		
	LC_{50} (ppm)	Lower Limit (ppm)	Upper Limit (ppm)
Cocoa Bean Shell Extract (Theobroma cacao L.)	787,14	701,14	864,41

Based on the probit analysis in Table 2, the LC_{50} of cocoa bean shell extract against A*edes aegypti* L. larvae was determined to be 787.14 ppm within a 24-hour exposure period, with a lower limit of 701.14 ppm and an upper limit of 864.41 ppm. The lower limit represents the minimum extract concentration capable of killing 50% of the test larvae, while the upper limit represents the highest concentration that still results in 50% mortality.

4. Discussion

Toxicity is an indication of the presence of toxic substances or poison in a compound, whether administered as a single dose or in combination with others (Mappasomba et al., 2019). In this research, toxicity testing was performed by immersing larvae in solutions containing different concentrations of cocoa bean shell extract. The extract exhibits toxic effects on A*edes aegypti* L. larvae, thereby showing its potential as a biolarvicide. The magnitude of the toxic effect depends on the content of secondary metabolite compounds present in the extract and the duration of exposure, which can lead to an accumulation of toxic substances in the larvae. Larvicidal toxicity is quantified by the LC_{50} value, which is the concentration required to kill 50% of the larvae within a specified period.

In this study, using probit analysis via SPSS, an LC_{50} of 787.14 ppm was obtained. Toxicity is generally interpreted as follows: a compound is considered highly toxic if LC_{50} is less than 30 ppm, toxic if LC_{50} is between 30 and 1000 ppm, and non-toxic if LC_{50} is greater than 1000 ppm (Herfayati et al., 2020).

Observations also revealed differences in the morphology of A*edes aegypti* L. larvae before and after treatment (Figure 1).

(a) Before (b) After

Figure 1 Morphological Differences of A*edes aegypti* L. Larvae Before and After Extract Treatment(4x10 Magnifcation)

There are morphological differences in the masquito larvae *Aedes aegypti* L. before and after treatment see Figure 1 Before treatment, larvae appeared normal, with intact body parts. While after treatment, several changes were observed: the body color turned pale, damage occurred to parts such as the eyes and antennae, thoracic bristles were detached, lateral hairs on the abdomen were lost, the respiratory tract was damaged, and the color of the siphon changed from brown to pale. Additionally, behavioral changes were noted. Initially, the larvae actively moved up and down along the water surface; post-treatment, the larvae became inactive, tended to sink, and several died.

Phytochemical screening confirmed that cocoa bean shells (Theobroma cacao L.) have potential as a botanical insecticide because they contain secondary metabolite compounds such as flavonoids, alkaloids, steroids, saponins, and tannins (Dewi et al., 2021). These compounds can enter the larval body through natural openings (via contact), through ingestion (as stomach toxins), and through the respiratory siphon. Each compound acts on specific organs in the larvae.

For example: flavonoids function as respiratory toxins by targeting the larval siphon, leading to damage that interferes with breathing (Sigit et al., 2022), alkaloids serve as both stomach and contact toxins that can degrade cell membranes through the cuticle or digestive tract (Bisyaroh, 2020), steroids may inhibit the larval molting process by causing abnormal deposition of chitin in the cell walls, thereby affecting growth (Suari et al., 2021), saponins act as contact toxins; when they come into direct contact with the larva's outer cuticle, their molecules gradually penetrate and are absorbed, eventually leading to death.

Saponins may also exhibit neurotoxic effects, causing spasms and movement impairment (Wahyuni, 2016), tannins disturb the digestive system by binding to proteins needed for larval growth and by reducing the activity of digestive enzymes, which disrupts cellular metabolism (Ayal et al., 2021). These compounds enter the larval body through natural openings (via contact), ingestion (stomach toxins), and the respiratory siphon to target specific organs.

5. Conclusion

Based on the research results, cocoa bean shell extract (Theobroma cacao L.) exhibits toxicity toward A*edes aegypti* L. larvae as evidenced by an LC50 value of 787.14 ppm (with a lower limit of 701.14 ppm and an upper limit of 864.41 ppm) after a 24-hour exposure period. This indicates that cocoa bean shell extract is toxic to A*edes aegypti* L. larvae, supporting its potential application as a biolarvicide.

Disclosure of conflict of interest

The authors declare no conflict of interest

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